## REMARKS

Claims 1, 4-7, and 16 were pending. Claims 1 and 4–7 have been canceled without prejudice. New claims 19-21 have been added. Accordingly, claims 16 and 19-21 are currently pending.

Support for new claims 19-21 can be found throughout the specification and claims as filed, *e.g.*, originally filed claims 5-6, respectively.

The foregoing claim cancellations and amendments should in no way be construed as an acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed, or other claims of equivalent or broader scope, in another application(s). No new matter has been added.

Attached hereto is a marked-up version of the changes made to the application by the current amendment. The attached page is captioned "Version with Markings to show changes made." Also attached is a copy of all the claims currently pending.

***Rejection of Claims 1 and 4-6 Under 35 U.S.C. § 112, First Paragraph)***

Claims 1 and 4-6 are rejected under 35 U.S.C. § 112, first paragraph, as failing to meet the written description requirement. Applicants respectfully traverse this rejection. However, to expedite prosecution, claims 1 and 4-6 have been canceled without prejudice. Therefore, this rejection is moot.

***Rejection of Claim 7 Under 35 U.S.C. § 102(e)***

Claim 7 is rejected under 35 U.S.C. § 102(e) "as being clearly anticipated by Gray *et al.* (U.S. Patent No. 5,447,841, filed 14 December 1990) in view of the teaching of Pinkel *et al.* (Proc. Natl. Acad. Sci. USA, 1988, 85:9138-9142)." Applicants respectfully traverse this rejection. However, to expedite prosecution, claim 7 has been canceled. Therefore, this rejection is moot.

***Rejection of Claim 7 Under 35 U.S.C. § 102(a)***

Claim 7 is rejected under 35 U.S.C. § 102(a) "as being clearly anticipated by Pinkel *et al.* (Proc. Natl. Acad. Sci. USA, 1988, 85:9138-9142)." Applicants respectfully traverse this rejection. However, to expedite prosecution, claim 7 has been canceled. Therefore, this rejection is moot.

***Rejection of Claims 1, 4, and 5 Under 35 U.S.C. § 103(a)***

Claims 1, 4, and 5 are rejected under 35 U.S.C. § 103(a) "as being unpatentable over Gray *et al.* (U.S. Patent No. 5,447,841, filed 14 December 1990) in view of the teaching of Pinkel *et al.* (Proc. Natl. Acad. Sci. USA, 1988, 85:9138-9142)." Applicants respectfully traverse this rejection. However, to expedite prosecution, claims 1, 4, and 5 have been canceled. Therefore, this rejection is moot.

***Rejection of Claims 1, 4, and 5 Under 35 U.S.C. § 103(a)***

Claims 1, 4, and 5 are rejected under 35 U.S.C. § 103(a) "as being unpatentable over Pinkel *et al.* (Proc. Natl. Acad. Sci. USA, 1988, 85:9138-9142)." Applicants respectfully traverse this rejection. However, to expedite prosecution, claims 1, 4, and 5 have been canceled. Therefore, this rejection is moot.

***Rejection of Claims 1, 4-7 and 16 Under 35 U.S.C. § 103(a)***

Claims 1, 4-7, and 16 are rejected under 35 U.S.C. § 103(a) "as being unpatentable over Grey *et al.* (U.S. Patent No. 5,446,841, filed 14 December 1990) and Smith *et al.* (Nature, 1986, 321:674-679) and Pinkel *et al.* (Proc. Natl. Acad. Sci. USA, 1988, 85:9138-9142)." Applicants respectfully traverse this rejection. However, to expedite prosecution, claims 1 and 4-7 have been canceled. Therefore, this rejection is moot as it pertains to claims 1 and 4-7.

Claim 16 is drawn to a method of determining over-representation or under-representation of a selected chromosome or a portion thereof in human tumor ***interphase*** cells comprising the steps of selecting a chromosome, or portion thereof, treating the human tumor interphase cells to render nucleic acid sequences present in the cells available for hybridization, combining the human tumor interphase cells with a hybridization mixture comprising labeled DNA fragments derived from the selected chromosome, competitor DNA, and nonhuman genomic DNA, under conditions appropriate for hybridization of complementary nucleic acid sequences to occur, and detecting labeled DNA fragments derived from the selected chromosome in order to determine the over-representation or under-representation of the selected chromosome, or a portion thereof, in human tumor interphase cells. Gray *et al.* fail to teach or suggest the claimed such method with sufficient description so as to enable one of ordinary skill in the art to practice the claimed methods without undue experimentation.

Gray *et al.* discuss that the success of any particular *in situ* hybridization methods depends upon several critical factors. In particular, Gray *et al.* state (at col. 11, lines 16-23) that

> [t]hree factors influence the staining sensitivity of a heterogeneous mixture of hybridization probes: (1) efficiency of hybridization (fraction of target DNA that can be hybridized by probe), (2) detection efficiency (*i.e.*, the amount of visible signal that can be obtained from a given amount of hybridization probe), and (3) level of noise produced by nonspecific binding of the probe or components of the detection system.

Gray *et al.* go on to discuss the numerous reagents and conditions that must be selected and optimized for any particular *in situ* hybridization technique (see col. 11, line 24 through col. 13, line 11). These include:

- type of fixative and fixation procedure;
- type of agent(s) for removing proteins and procedure for deproteinization;
- procedure for removal of residual RNA from fixed chromosomes;
- conditions for denaturation of chromosomal DNA so that probes can gain access to complementary single stranded regions;
- procedure for removal of denaturing agents;
- conditions for application of the heterogeneous probe mixture which allow the probes to anneal to complementary sites (*i.e.*, hybridization conditions);
- selection of the concentration of probes in the heterogeneous mixture;
- procedures for reducing non-specific binding of probe DNA;
- conditions for posthybridization washes to remove probe not bound to specific hybrids;
- conditions for detecting the hybridized probes.

While Gray *et al.* discuss these factors and provide some guidance as to reagents and conditions that may be used, Gray *et al.* admit numerous times throughout the specification that further experimentation may be required for particular applications. For example, Gray *et al.* state that

> [t]he following comments are meant to serve as a guide for applying the general steps listed above. Some experimentation may be required to establish optimal staining conditions for particular applications [see col. 11, lines 36-39] . . .
>
> Optimization of deproteinization requires a combination of protease concentration and digestion time that maximize hybridization, but does not cause unacceptable loss of morphological detail. Optimum conditions vary according to chromosome types and method of fixation.

> Thus, for particular applications, some experimentation may be required to optimize protease treatment [see col. 11, line 63 to col. 12, line 2]. . .
>
> Determination of the optimal incubation time, concentration, and temperature within these ranges [for hybridization conditions] depends on several variables, including the method of fixation and the chromosome type [see col. 12, lines 27-30]. . .
>
> Optimal hybridization conditions for particular applications depend on several factors, including salt concentration, incubation time of chromosomes in the heterogeneous mixture, and the concentrations, complexities and lengths of the probes making up the heterogeneous mixture. Roughly, the hybridization conditions must be sufficiently denaturing to minimize nonspecific binding and hybridizations with excessive numbers of base mismatches. On the other hand , the conditions cannot be so stringent as to reduce hybridizations below detectable levels or to require excessively long incubation times [see col. 12, lines 42-55].

For application of *in situ* suppression hybridization in interphase cells which uses competitor DNA, Gray *et al.* provide no specific guidance with regard to the myriad factors discussed above. Gray *et al.* disclose a single exemplification of chromosome-specific staining using competitor DNA containing repetitive sequences (see Paragraph VI at col. 15, line 58 through col. 16, line 57) and this exemplification uses ***metaphase cells***. The specification provides no exemplification of interphase cells, no guidance as to whether the conditions used for metaphase cells are applicable to interphase cells, or whether certain conditions should be altered and, if so, how to alter them. At most, the Gray *et al.* patent provides merely a starting point for further experimentation on *in situ* suppression hybridization in interphase cells, using competitor DNA containing repetitive sequences.

In addition to the Gray *et al.* disclosure itself, there is additional evidence in the art that the ordinarily skilled artisan would not believe that Gray *et al.*'s exemplification of *in situ* suppression hybridization in metaphase cells would enable the method in interphase cells, without further experimentation. In particular, Applicants draw the Examiner's attention to Landegent *et al.* (1987) *Human Genetics* 77:366-370 (of record as reference "CP"). Landegent *et al.* teach *in situ* hybridization in metaphase cells using as probes one or more cosmids of the human Tg gene. Landegent *et al.* (at page 367, column 2, second paragraph of the Results section) describe the probes used in their method as follows:

> four different randomly chosen cosmids of the human Tg gene (of which the chromosomal localization is known, Baas *et al.* 1985; Landegent *et al.* 1985b) were used (Van Ommen *et al.* 1983; Baas *et al.* 1986) . . . These cosmids have not been characterized in the actual number and type of repeats present, but all gave a strong overall staining when hybridized to total human DNA under standard conditions.

Landegent *et al.* teach that the method described therein is not applicable to interphase cells. In particular, Landegent *et al.* (at page 369, column 2, last paragraph) state:

> The method described here can extend the applicability of non-radioactive procedures since it elegantly bypasses the lower sensitivity problem, obviating subcloning of a minimal amount of unique parts required. When, in time, the sensitivity has reached a level allowing the detection of small (1-2 kb) single-copy sequences on metaphase chromosomes on a routine basis, the described procedure may still retain its attractiveness for several purposes. For example: . . . Detection of chromosomal aberrations in prenatal diagnosis, for example a trisomy, in interphase nuclei in microscopic slides (Cremer *et al.* 1986) or through hybridization in suspension and flow cytometry. In these cases it would be favorable to use a whole panel of chromosome-specific cosmids instead of a small unique probe or a cloned alphoid sequence to ensure strong hybridization signals with great specificity, for a more reliable diagnosis [emphasis added].

The above-quoted passage contains the only reference to interphase cells, or nuclei thereof, in Landegent *et al.* and it is entirely future-oriented ("when, in time..."). Thus, Landegent *et al.* clearly teach that the sensitivity of their method (*i.e.*, *in situ* suppression hybridization in ***metaphase cells***) is not sufficient for application to interphase cells. Accordingly, since Gray *et al.* essentially exemplify the same method as Landegent *et al.*, namely, *in situ* suppression hybridization in ***metaphase cells***, and Landegent *et al.* teach that this method is not applicable to interphase cells, or nuclei thereof, without further experimentation, the ordinarily skilled artisan would not believe that Gray *et al.* had enabled this method for interphase cells.

In contrast to Gray *et al.*, Applicants have enabled a method of determining over-representation or under-representation of a selected chromosome in human tumor ***interphase cells*** and exemplified this method in different cell types and using probes for detecting different target chromosomal DNAs, thereby demonstrating the general applicability of the claimed method. For example, in Example 1 (see, *e.g.*, Figure 5),

Applicants successfully detect chromosomes 1, 7 and 18 in normal lymphocyte interphase nuclei and in Example 2 (see, *e.g.*, Figures 10B-10F), Applicants successfully detect chromosomes 1, 7 and 18 in interphase nuclei of tumor cells (glioma cells). Other successful demonstrations of the method in interphase cells, are found throughout the specific examples of the invention.

Since Gray *et al.* fail to enable the claimed method, claim 16 is patentable in view of Gray *et al.*

Further, neither Smith *et al.* nor Pinkel *et al.* cure the deficiencies of Gray *et al.*

Like Gray *et al.*, Smith *et al.* and Pinkel *et al.* fail to teach or suggest the claimed method with sufficient description so as to enable one of ordinary skill in the art to practice the claimed methods without undue experimentation. The arguments set forth above are reiterated here and apply equally to Smith *et al.* and Pinkel *et al.* Accordingly, the claims are patentable in view of the cited references.

**SUMMARY**

In view of the amendments and remarks set forth above, it is respectfully submitted that this application is in condition for allowance. If there are any remaining issues, the Examiner is invited to call the undersigned at (617) 227-7400.

Respectfully submitted,
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**Version with Markings to Show Changes Made**

In the Claims:

Claims 1 and 4-7 have been canceled.

New claims 19-21 have been added as follows:

19. (New) The method of claim 16, wherein the labeled DNA fragments are selected from the group consisting of probes comprising total recombinant library DNA, probes comprising DNA inserts purified from a chromosome-derived recombinant DNA library, and probes comprising specific DNA fragments derived from chromosomes.

20. (New) The method of claim 16, wherein the labeled DNA fragments are selected from the group consisting of DNA fragments labeled with at least one fluorochrome, DNA fragments labeled with at least one member of a specific binding pair, and DNA fragments labeled with an enzyme.

21. (New) The method of claim 20, wherein the fluorochrome is selected from the group consisting of fluorescein, rhodamine, Texas red, Lucifer yellow, phycobiliproteins and cyanin dyes.